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ASSOCIATION BETWEEN RETINOL METABOLISM AND DIABETIC RETINOPATHY

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The purpose of this article was to study the possible interaction and relation between vitamin A status in diabetic retinopathy and the state of proliferative changes occurring in these cases. Twenty five healthy and sixty diabetic retinopathy patients were included in this study. Diabetic patients were classified into four groups according to the type of diabetic retinopathy. Group 1: normal fundus; Group 2: proliferative; Group 3: non-proliferative, and Group 4: hard with exudate. Groups were further classified into 3 subgroups according to the route of treatment; whether receiving insulin therapy alone or oral hypoglycemic tablets or both.

The level of serum glucose, insulin, retinol binding protein (RBP), vitamin A and β-carotene were estimated for all groups.

The fasting blood glucose and glycosylated hemoglobin levels of patients suffering from diabetic retinopathy were high in all diabetic groups. The highest levels were noticed among proliferative group. Cases with normal fundus receiving insulin therapy had the lowest levels of blood sugar. Serum insulin level was markedly high in all diabetic groups when compared within control values, markedly higher among those who received oral hypoglycemic tablets alone. Serum vitamin A and β-carotene levels were significantly decreased in all groups of patients, the lowest was attained for proliferative group receiving insulin. The level of RBP was significantly high in all diabetic groups. Serum RBP was markedly low in the subgroup of patients under insulin therapy.

It was concluded that the patients with diabetic retinopathy included in this study suffer from a state of non controlled hyperglycemia which induces the generation of free radicals. This state together with decreased level of the antioxidant, β-carotene or vitamin A results in a state of retinal damage. The deficiency of vitamin A is believed to participate in the phenomena of retinal cell proliferation.

INTRODUCTION

Patients suffering from diabetes type I or II are classified among the groups at risk of low vitamin A or the pro-vitamin A compound known as β-carotene [Sasaki *et al.*, 1995; Tuitoek *et al.*, 1996; Abahusain *et al.*, 1999]. Photoreception in the eye is the function of two specialized cell types located in the retina; the rod and cone cells. Both rod and cone cells contain photoreception pigment in their membranes composed of a protein called opsin to which an aldahyde of vitamin A is covalently coupled. It is thus clear that retinol is needed to maintain the visual process through protection of the retina from different hazards that affect the eye. β-carotene which is known for its antioxidant property protects different tissues including the retina from degeneration caused by active free radicals. Vitamin A is transported in the circulation by specific retinol binding protein (RBP). This protein is responsible for distribution of vitamin A to different tissues including the retina [Loredana *et al.*, 1999].

Retinopathy is the most important ocular manifestation of diabetes and is characterized by background retinopathy or proliferative retinopathy where new blood vessel develops in and anterior to the retina. Vitamin A or β-carotene deficiency are expected to contribute to the changes that affect the retina in diabetic retinopathy since vitamin A is essential for normal cellular growth and deficiency, while β-carotene can protect against hazards of hyperglycaemia that occurs in diabetic patients and initiate cell proliferation in retinopathy [Augustin *et al.*, 2002; Zobali *et al.*, 2002].

The aim of this article was to study the possible interaction and relation between vitamin A status in diabetic patients and the state of proliferative changes occurring in cases suffering from retinopathy.

SUBJECTS AND METHODS

The present study was done on a group of patients suffering from diabetic retinopathy. Sixty patients were selected from those attending the out-patient, Ophthalmic Clinic Institute of Ophthalmology. They were excluded from any complications other than retinopathy. Complete eye examination was done to each case. Fundus examination using slit lamp biomicroscopy and + go diopter wolk lens. Fluoresce fundus angiography was done when needed. Twenty five healthy subjects were included in this study as control.

The diabetic patients were classified into four groups according to the type of diabetic retinopathy as follows:

Group 1: fifteen diabetic patients with normal fundus, their mean age 54.4±3.08 years.

Group 2: fifteen cases with non-proliferative diabetic retinopathy, the mean age 51.7±1.86 years.

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Group 3: Twelve cases with proliferative diabetic retinopathy, the mean age 54.7±2.42 years.

Group 4: Eighteen cases with hard exudative diabetic retinopathy, the mean age 56.7±1.85 years.

Twenty five healthy subjects with more or less similar sex, age and distribution were included to serve as controls.

Furthermore, the cases were classified according to the route of management into three subgroups as follows:

Group A: those who were under insulin therapy alone [(1 mL suspension contains 40 IU of human monocomponent insulin (Biosynthetic, 30% as soluble insulin and 70% as isophane insulin; Novo Nordisk AIS, 2880 Bagsveard, Denmark)]. The dose was 30 Iu/d.

Group B: patients receiving oral hypoglycemic tablets (Metaformine, 500 mg/d; Seid, Egypt & Glibinclimid, 5mg/d; EG, Frankfurt-Main – Germany).

Group C: those who were receiving insulin and oral hypoglycaemic tablets.

Blood samples were collected after overnight fast and divided into two portions; one portion was collected on heparin; the second portion was clotting. The two portions were centrifuged at 3500 rpm for 10 min; the serum and plasma were separated and frozen at -30°C until analysed. Vitamin A and β–carotene were analysed within one week. Vitamin A and β-carotene were estimated according to the method of Neeld and Pearson [1963]. Serum insulin assay was done using a competitive Enzyme Linked Immunosorbent Assay (ELISA) kit, using human insulin as standard by the method of the manufacturer's instructions (Insulin, Biosource ce Europe S.A., Nivelles. Belgium). Retinol binding protein assay was carried out using radioimmunodiffusion technique by the method of the manufacturer's instructions (Retinol binding protein, the binding site Ltd. Birmingham, UK). Blood sugar was estimated enzymatically according to the method of Siest *et al.* [1981] as cited by BioMerieux. The glycosylated hemoglobin was estimated according to the method of Sudhakar Nayak and Pattabiraman [1981] as cited by Stabno Laboratory.

Dietary intake. A 24-h dietary recall was done to each diabetic patient and to control. The dietary intake of carbohydrates, proteins from animal and plant source, fat, total energy and vitamin A were evaluated. Nutrient intake was calculated from the food composition table [Gordon *et al.*, 1994].

Statistical analysis. SPSS package (version 10) was used for data analysis. Mean and standard error were descriptive measures of quantitative data. Pearson correlation test was used to measure correlation between variables. P values always 2 tailed and significant if <0.05.

RESULTS

Table 1 shows the results obtained from the analysed parameters of the patients in the different groups. As shown in the table the level of fasting blood glucose ranged from 171.4±12.3 to 284.8±29.5 mg/dL. The serum insulin level was markedly high in all groups, it ranged between 10.9±1.87 to 13.51±2.04 μLu/mL.

The serum vitamin A level was significantly decreased in all groups of patients, the lowest value 13.8±1.5 μg/dL was attained for the group of non-proliferative retinopathy. The serum carotene level was also markedly low in all groups. The values reported for β-carotene ranged from 49.0±6.8 to 80.3±8.7 μg/dL relative to a normal value of 121±5.3 μg/dL. The level of serum RBP was markedly high in all groups of patients suffering from retinopathy, it ranged from 58.9±4.12 mg/L in the cases of non-proliferative group to 81.9±6.88 mg/L in the proliferative group compared to a normal value of 51.1±3.11 mg/L.

Figures 1, 2, 3 and 4 show the value of the analysed parameters when the groups of patients were classified according to the route of management into three groups whether those receiving insulin alone, hypoglycaemic tablets or both insulin and hypoglycaemic tablets. According to this classification, it is noticed that in the case of patients suffering from diabetic with normal fundus (Figure 1), the blood glucose level of those given insulin was the lowest (127±9.8 mg/dL) followed by the group given both routes of treatment then those given the hypoglycaemic tablets. There was no significant difference of the glycosylated hemoglobin level between subgroups. The serum insulin level of the subgroup receiving insulin therapy was the lowest among subgroups of this group followed by the subgroup receiving both treatments than those receiving oral tablets. The serum vitamin A level and β-carotene were the lowest among the subgroups. The serum RBP was markedly low in the subgroup of patients under insulin therapy.

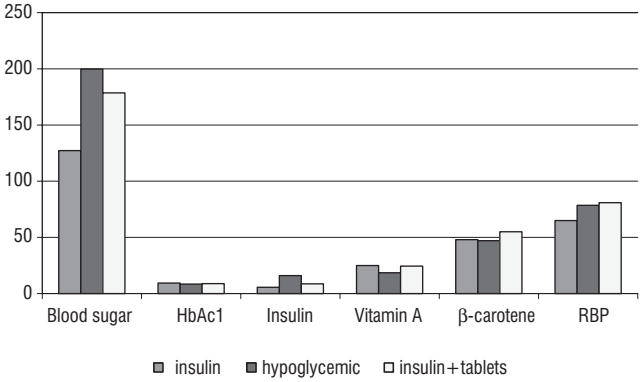

FIGURE 1. Blood sugar, glycosylated haemoglobin, insulin, vitamin A, β-carotene and retinol binding protein levels of normal fundus group according to the route of management.

In the group of non-proliferative retinopathy (Figure 2), it was noticed that the serum RBP level was still markedly high in the subgroups. The serum insulin was about three times the normal value in both subgroups, either those under insulin therapy or the subgroup given the hypoglycaemic tablets. The serum vitamin A was markedly reduced in the three subgroups. The most significant reduction was noticed in the subgroup receiving oral hypoglycaemic tablets. There were no marked differences between changes in the glycosylated hemoglobin and serum β-carotene or RBP among different subgroups.

Regarding the proliferative group (Figure 3), all groups showed a markedly high blood glucose level. The level of serum insulin in the group receiving the oral tablets 22.0±5.0 μLu/mL was about double that 11.6±3.22 μLu/mL of the subgroup given insulin and more than double that 8.3±4.37 μLu/mL of the subgroup given both routes. The serum vitamin A and β-carotene levels were markedly

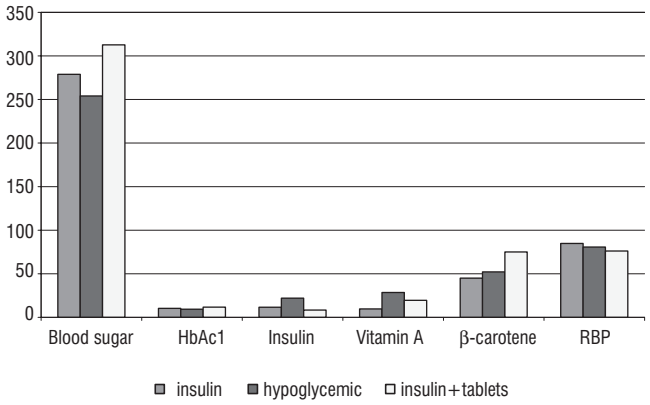

FIGURE 2. Blood sugar, glycosylated haemoglobin, insulin, vitamin A, β-carotene and retinol binding protein levels of proliferative group according to the route of management.

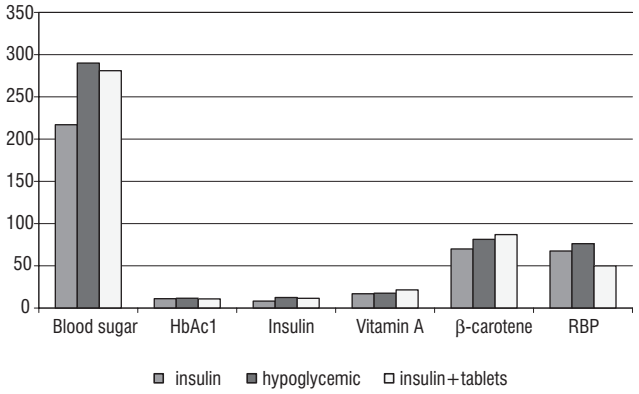

FIGURE 3. Blood sugar, glycosylated haemoglobin, insulin, vitamin A, β-carotene and retinol binding protein levels of non-proliferative group according to the route of management.

decreased in the subgroup given insulin therapy compared to other subgroups and also to the normal group. The serum RBP was high and more or less similar within the three subgroups.

In the case of the exudative retinopathy group (Figure 4), it was noticed that the blood glucose glycosylated hemoglobin levels were markedly high in all subgroups. The values reported were 217±32.5 mg/dL for the group receiving insulin, 290±84.6 mg/dL for the group given hypoglycaemic tablets and 281±56.1 mg/dL for the group receiving both routes of management. There was no marked difference

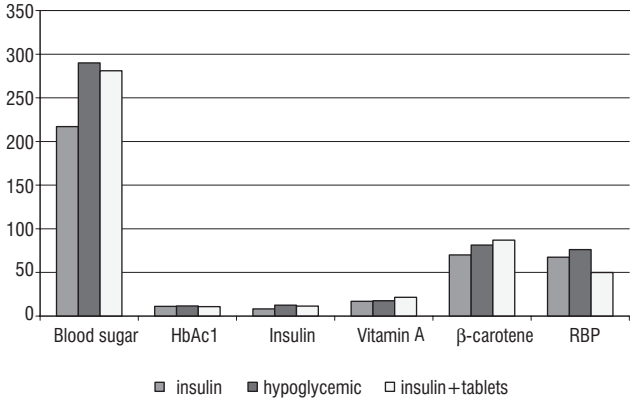

FIGURE 4. Blood sugar, glycosylated haemoglobin, insulin, vitamin A, β-carotene and retinol binding protein levels of exudates group according to the route of management.

between serum insulin level in both subgroups given the oral hypoglycaemic tablets or those receiving both routes of treatment. The level in both of these subgroup was markedly high. The level of serum insulin in the subgroup receiving insulin therapy was also higher than normal but lower than other two subgroups. The serum vitamin A and β-carotene among these different subgroups were more or less similar. The highest increase in the RBP was noticed in the subgroup given oral hypoglycaemic tablets followed by those given insulin therapy then those put under both routes of treatment.

DISCUSSION

As mentioned, patients included in this study were selected among those attending the Ophthalmic Clinic, Institute of Ophthalmology who were diabetic and suffering from retinopathy with all types. Analysis of the parameters of those patients (Table 1) shows that all groups of patients had a significantly high blood glucose and glycosylated hemoglobin levels ranging from 171.4±13.2 mg/dL and 8.6% respectively in the case of diabetic patients with normal fundus to 284.8±29.5 mg/dL and 10.8% respectively for diabetic patient with hard exudative. This means that most of those patients had no perfect control of their blood glucose and glycosylated hemoglobin levels, in spite of the different routes of treatment they follow. The significant rise in blood glucose and glycosylated hemoglobin levels of those diabetic patients may explain the development of complication in the retina. Retinal changes and proliferative diabetic retinopathy have been reported to be a complication of the uncontrolled blood glucose level in diabetics [Edward, 1976].

Serum insulin level of the different groups of patients suffering from diabetic retinopathy included in this study was found to be significantly higher than the control value. The mean value reported for the different groups was 2.20% higher in diabetic cases with normal fundus, 2.48.0% higher in cases with proliferative diabetic retinopathy, 2.68% in cases with non-proliferative diabetic retinopathy and 2.16% in cases with hard exudates. These data show that serum insulin concentration in all groups of patients was even above normal values which categorized those patients as type II diabetics. However, according to the route of treatment many of those cases were receiving insulin injection for the control of blood glucose level. According to the history taken from those patients some of them (n=17) started treatment of the diabetic state with oral hypoglycemic tablets to control hyperglycemia. Some other cases (n=17) among those included in this study received oral hypoglycaemic tablets and insulin injection to control hyperglycaemia. The rest of the patients were depending mainly on insulin injection (n=26).

These results show that, diabetic patients type II may at certain stage of the disease be oriented and not able to depend on their own insulin alone. It is known that in type II diabetes there are several reasons why insulin is not functioning, among these reasons are the absence of insulin receptors [Accili *et al.*, 1989; Kadowaki *et al.*, 1990; Basual *et al.*, 1997], auto-immune-reaction against native insulin [Kahn *et al.*, 1976, 1980; Taylor *et al.*, 1989], and defective insulin structure [Taylor *et al.*, 1990 a, b; Granado *et al.*, 1998]. According to this, our cases may be categorized to

TABLE 1. The mean value of fasting blood sugar, glycosylated hemoglobin, insulin, retinol binding protein, vitamin A and β-carotene, in control and different types of diabetes.

		Hb Ac_1 (%)	Blood sugar mg/dL	Insulin (μLu/mL)	Vitamin A (μg/dL)	β-carotene (μg/dL)	RBP (mg/L)
Control N=25 (Group 1)	Mean	6.6	90.9	5.04	36.1	121.0	51.1
	±S.E.	0.09	4.36	0.23	1.59	5.30	3.11
Normal fundus N=15 (Group 2)	Mean	8.6	171.4	11.08	21.9	49.0	74.5
	±S.E	0.026	12.3	1.95	2.91	6.80	6.10
	P_1<	0.012*	0.0001*	0.0001*	0.0001*	0.0001*	0.001*
Proliferative N=12 (Group 3)	Mean	10.5	283.1	12.5	15.3	53.8	81.9
	±S.E	0.46	13.2	2.51	2.41	7.49	6.86
	P_1<	0.016*	0.0001*	0.0001*	0.0001*	0.0001*	0.001*
	P_2<	0.004	0.0001*	n.s.	n.s.	n.s.	n.s.
Non-proliferative N=15 (Group 4)	Mean	10.3	193.7	13.51	13.8	64.3	58.9
	±S.E	0.45	20.10	2.04	1.50	9.10	4.12
	P_1<	0.016*	0.001*	0.0001*	0.001*	0.001*	n.s.
	P_2<	0.002	0.001*	n.s.	0.02*	n.s.	0.044*
Exudates N=18 (Group 5)	Mean	10.8	284.8	10.9	20.5	80.3	66.5
	±S.E.	0.37	29.5	1.87	2.7	8.7	4.91
	P_1<	0.017*	0.001*	0.0001*	0.0001*	0.001*	0.008*
	P_2<	0.005	0.02*	n.s.	n.s.	0.01*	n.s.

either the group having self immune-reaction against insulin or those with defective structure of the molecule. This is logic, since those patients responded to injection with external insulin.

It is not easy to confirm a relation between both blood glucose and/or glycosylated hemoglobin levels and serum insulin of those patients and retinal changes associating diabetes. This is because diabetic patients with normal fundus also had a serum insulin level about double the normal value, however their fasting blood glucose 171.4±12.3 mg/dL and glycosylated hemoglobin 8.6±0.026 % were markedly lower than other diabetic groups suffering from retinal changes. It is clear that the route of treatment followed by patients in this group whether it is based on oral hypoglycaemic tablets or insulin injection or both is effective to realize reasonable control of blood glucose level which is believed to be contributable to the absence of retinal changes in those diabetic patients.

In addition to the role of hyperglycaemia in the development of retinal changes in diabetic patients, there has been reported an effective role of β-carotene and vitamin A in the protection of the retina against this hazard [Polidori *et al.*, 2000; Abahusain *et al.*, 1999; Krempt *et al.*, 1991]. The data reported in this study show that all groups of diabetic patients even those with normal fundus had a significantly lower level of serum of β-carotene and vitamin A compared to normal control.

A decreased level of serum vitamin A was several times reported before in diabetic patients particularly with type I diabetes [Lu *et al.*, 2000; Polidori *et al.*, 2000; Tuitoek *et al.*, 1996]. Our data proved that the deficiency of vitamin A can occur in both types of diabetes whether type I or II. The reason for this low vitamin A level in diabetics was attributed to either deficiency of intake [Mustafa *et al.*, 1995], defect in transformation of β-carotene to vitamin A [Basu & Basualdo, 1999] or defect of metabolic processes concerned with vitamin A transport by the RBP [Murray, 1996]. According to the dietary recall made in this study, the intake of vitamin A was not significantly different from that of normal controls in most groups of patients. Only there was a decrease in vitamin A intake by the non-proliferative group. This means that the drop in vitamin A level of this group suffering from retinopathy is not due to dietary deficiency. In addition the RBP of the different groups of patients was significantly higher than normal, which means that there is no defect in circulation of retinal, if present, among different organs including the eye.

It is most probable that the normal intake of vitamin A of those diabetic patients is in the form of β-carotene, since those patients are from the low socioeconomic sector of the population who usually have limited intake of animal food that contains vitamin A [Nerup *et al.*, 1988]. It is thus suggested that there is a defect in the transformation of the carotene to vitamin A in diabetic patients as suggested by some authors [Dina *et al.*, 2002; Basu & Basualdo, 1999].

When cases in the different groups were classified according to the route of management (Figure 1, 2, 3 and 4), it was noticed that the cases treated with insulin alone were having more or less the lowest serum insulin value relative to those given hypoglycaemic tablets or both insulin and hypoglycaemic tablets. This seems reasonable and convenient with similar findings reported for this condition [Bach *et al.*, 1988; Yalow & Berson, 1960]. There were also found significant changes in the serum vitamin A and β-carotene levels among subgroups particularly in the group of diabetic patients with proliferative retinopathy where the subgroup depending on insulin therapy alone were having the most decreased serum levels of vitamin A and β-carotene. Such pattern of changes was not consistent in other groups (Table 2).

TABLE 2. Nutrient consumption of control and different types of diabetic retinopathy.

Groups		Energy Kcal	Proteins		CHO (g)	Fats (g)	Vitamin A (RI)
			Animal (g)	Plant (g)			
Control	Mean	2179.4	31.9	44.2	396	53.9	807.9
	±S.E.	165.7	4.18	2.93	26.5	5.8	46.6
Normal funds	Mean	2129.3	35.7	56.7	332.5	48.6	645.7
	±S.E	214.1	6.03	8.9	33.4	8.3	125.4
	P<	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Proliferative	Mean	2113.5	34.0	54.2	325.8	67.4	719.7
	±S.E	199.5	9.01	8.84	56.9	12.8	193.2
	P<	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Non-proliferative	Mean	2626.4	58.8	32.5	314.6	42.6	463.9
	±S.E	105.6	5.15	5.05	50.8	6.64	67.7
	P<	0.05	0.01	n.s.	n.s.	n.s.	0.001
Exudates	Mean	1652.7	34.0	44.0	352.3	54.8	723.1
	±S.E	95.4	5.4	4.0	40.9	6.03	64.6
	P<	0.05	n.s.	n.s.	n.s.	n.s.	n.s.

Vitamin A is essential to inhibit neovascularization and proliferation of human retinal pigment epithelial cells [Schonfeld, 2000]. It is thus expected that the deficiency of vitamin A participate in the occurrence of retinal changes among those patients. Although our data confirm a relation between vitamin A deficiency and occurrence of diabetic retinopathy, the extent of deficiency and type of retinopathy have not been recognised yet. It is suggested that the deficiency of vitamin A is due to non efficient transformation of β-carotene.

CONCLUSIONS

It was concluded that diabetic patients included in this study suffer from hyperglycaemia which induces the generation of the destructive free radicals together with a decreased level of the protective factors namely β-carotene and vitamin A which results in this state of retinopathy. The significantly low level of β-carotene in those patients still suggests that there is a defect also in β-carotene absorption since the intake of this carotenoid is normal. It is thus recommended that the route of management of those patients should insure keeping blood glucose level within normal range and supplying excessive amounts of antioxidant vitamins including β-carotene and vitamin A, vitamin C and E. Vitamin A supplement should be given to overcome the problem of defective transformation of β-carotene.

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